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Increased Expression of Low-Density Lipoprotein Receptors in a Smith-Lemli-Opitz Infant With Elevated Bilirubin Levels

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We report on an infant girl with severe RSH or Smith-Lemli-Opitz syndrome with hyperbilirubinemia. The infant died at age 2 months. Sterol analysis of liver and brain tissues showed marked elevations of 7-dehydrocholesterol with decreased levels of cholesterol. Immunocytochemical analysis demonstrated remarkable increases in low-density lipoprotein (LDL) receptors in these tissues, indicative of a deficiency in available cholesterol for tissue needs. Am. J. Med. Genet. 68:294–299, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: Smith-Lemli-Opitz syndrome; low density lipoprotein; receptor; bilirubin

INTRODUCTION

The RSH or Smith-Lemli-Opitz syndrome (SLOS) is an autosomal-recessive disorder of multiple congenital anomalies due to defective cholesterol biosynthesis. The incidence of this disease has been estimated at 1:20,000–1:40,000 [Lowry and Yong, 1980]. Tint et al. [1994] reported that concentrations of the cholesterol precursor, 7-dehydrocholesterol, were elevated more than 2,000-fold above normal in serum and tissues from SLO patients, while plasma cholesterol levels were markedly lower, suggesting that 7-dehydrocholesterol Δ^7 reductase activity was defective. Subsequently, Salen et al. [1995] demonstrated decreased levels of 7-dehydrocholesterol Δ^7 reductase activity in liver microsomes from 4 SLOS patients. The clinical problems of these patients appear to arise from both a deficiency in available cholesterol and an excess of 7-dehydrocholesterol.

Patients with SLOS typically present with microcephaly, a narrow high forehead, strabismus, ptosis, apparently low-set, posteriorly-angulated ears, broad anteverted nares, micrognathia, cleft palate, and cataracts [Irons et al., 1994, 1995]. Additionally, these patients typically have low serum cholesterol levels, syndactyly of toes 2 and 3, polydactyly (generally postaxial), prenatal growth retardation, impaired postnatal growth, delayed myelinization, mental retardation, delayed motor development, congenital heart disease, sensitivity to light, deafness, cryptorchidism or hypospadis in males, renal cysts, adrenal enlargement, and hepatomegaly. In one infant, jaundice was reported [Curry et al., 1987].

In this report, we present our findings in a case of severe SLOS presenting with jaundice. Analysis of liver and brain sterols showed marked elevations of 7-dehydrocholesterol together with low levels of cholesterol. Immunohistochemical staining demonstrated increased expression of low density lipoprotein (LDL) receptors.

MATERIALS AND METHODS

Cholesterol and 7-dehydrocholesterol levels were determined by reverse-phase HPLC analysis. A weighed amount of tissue (about 100 mg) was saponified in 1 ml of 20% potassium hydroxide in 66% methanol in a boiling water bath for 30 min. Cholesterol, [^{3}H]-labeled, 4 pmol containing 20,000 cpm, was added as a recovery standard. After cooling to room temperature, samples were extracted four times with 2 ml each time of petroleum ether. The combined extracts were taken to dryness under a stream of argon. The residue was dissolved in 1 ml of methanol. A sample of 100 µl was resolved on a Spheri-5, RP-18, 5-µ reverse-phase column (Alltech Associates, Deerfield, IL). The eluate was monitored at A_{210}, and the mass of sterols was determined by comparison to standards. Identification of sterols was made by comparison to elution times for known standards. The recoveries of [^{3}H] cholesterol ranged from 80–97%. Values for cholesterol are expressed as mg $\times$ g^{-1} of tissue. This method gives comparable results to previously described methods for the determination of 7-dehydrocholesterol [Sattler et al., 1995; Tint et al., 1995; Batta et al., 1995].

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Histological studies were carried out on 5-μ-thick sections of paraffin-embedded tissues. Sections were stained with hematoxylin and eosin or with periodic acid-Schiff reagent. Magnifications are stated in figure legends. For detection of LDL receptor protein, 5-μ-thick sections of liver and brain from patient K.S. (see Clinical Summary, below) and an age- and sex-matched control were incubated with a 1:150 dilution of anti-rat LDL receptor sera, prepared as described previously [Ness and Zhao, 1994]. Detection was by a horseradish peroxidase-linked avidin-biotin system.

CLINICAL SUMMARY

K.S., a white girl, was born by cesarean section because of oligohydramnios at 35 weeks of gestation, with suspected multiple congenital anomalies. There was ultrasound evidence of intrauterine growth retardation. The infant was intubated for poor respiratory function. She was hypotonic, appeared to be microcephalic, had apparently low-set, posteriorly-angulated ears, short upturned nose, single palmar creases, postaxial polydactyly of the hands, and bilateral syndactyly of the second and third toes. Retrognathia was evident. There appeared to be a cleft of the soft palate. The hips appeared to be dislocated. She had congenital heart disease consisting of right ventricular dysfunction and respiratory distress syndrome. Jaundice was noted on the first day of life, and treatment with phototherapy was instituted and then discontinued after 6 days of life. However, the jaundice progressed and became severe. The total bilirubin reached 25–27 mg/dl, with a direct acting bilirubin of 7–8 mg/dl. Metabolic acidosis developed. The infant was also noted to be anemic and developed hepatomegaly with elevated liver enzyme levels. A renal ultrasound study showed what appeared to be a malrotated left kidney. The baby had episodes of sepsis, and exhibited feeding intolerance with upper gastrointestinal bleeding. The condition progressed and the child required respirator assistance. Death occurred at age 2 months.

PATHOLOGICAL STUDIES

At autopsy, this infant (Fig. 1) measured 43.5 cm in crown–heel length, and 30.5 cm in crown–rump length, with a head circumference of 29.5 cm and weight of 1,870 g, indicative of intrauterine growth retardation and microcephaly. The sclerae were noted to be deep yellow. A cleft palate, anteverted nostrils, apparently low-set and posteriorly angulated ears, postaxial hexadactyly of the upper limbs with short thumbs on each hand, bilateral syndactyly of the second and third toes, and a small sacral dimple were noted on external examination.

The heart weighed 40 g (normal, 20 g). There was biventricular hypertrophy, a patent ductus arteriosus, coarctation of the aorta, and bicuspid aortic valve with aortic valve dysplasia. Myocardial hypertrophy was present in sections from right and left ventricles.

Fig. 1. Patient K.S. at age 2 months. Microcephaly, apparently low-set, posteriorly-angulated ears, and severe jaundice were present.

The liver weighed 120 g (normal, 78 g). The liver was deep yellow and after fixation assumed a dark green color (Fig. 2A). The extrahepatic biliary ducts were intact. Microscopic sections of the liver showed severe cholestasis (Fig. 2B,C) of the hepatocytes, a distorted hepatic architecture, early septal fibrosis, and mild extramedullary hematopoiesis. Depositions of bilirubin and iron were present within hepatocytes. The anti-α-1 antitrypsin immunoperoxidase stain was negative.

The brain weighed 250 g (normal, 330 g). Examination of the brain, after formalin fixation, showed marked yellow bile staining of the meninges and bilirubin staining, particularly of the dura and cranial bones. There was a strikingly abnormal gynal pattern (Fig. 3A). Coronal sections demonstrated mild hydrocephalus with porencephaly, absence of the corpus callosum, and a small, hypoplastic cerebellum. Bile staining was present in the basal ganglia and dentate nucleus of the cerebellum, consistent with kernicterus (Fig. 3B). Sections of the brain through the cortex corresponding to the grossly abnormal gynal pattern showed abnormal neuronal migration with four instead of six cortical layers (Fig. 3C). A severe lack of myelination was also evident (Fig. 4D).

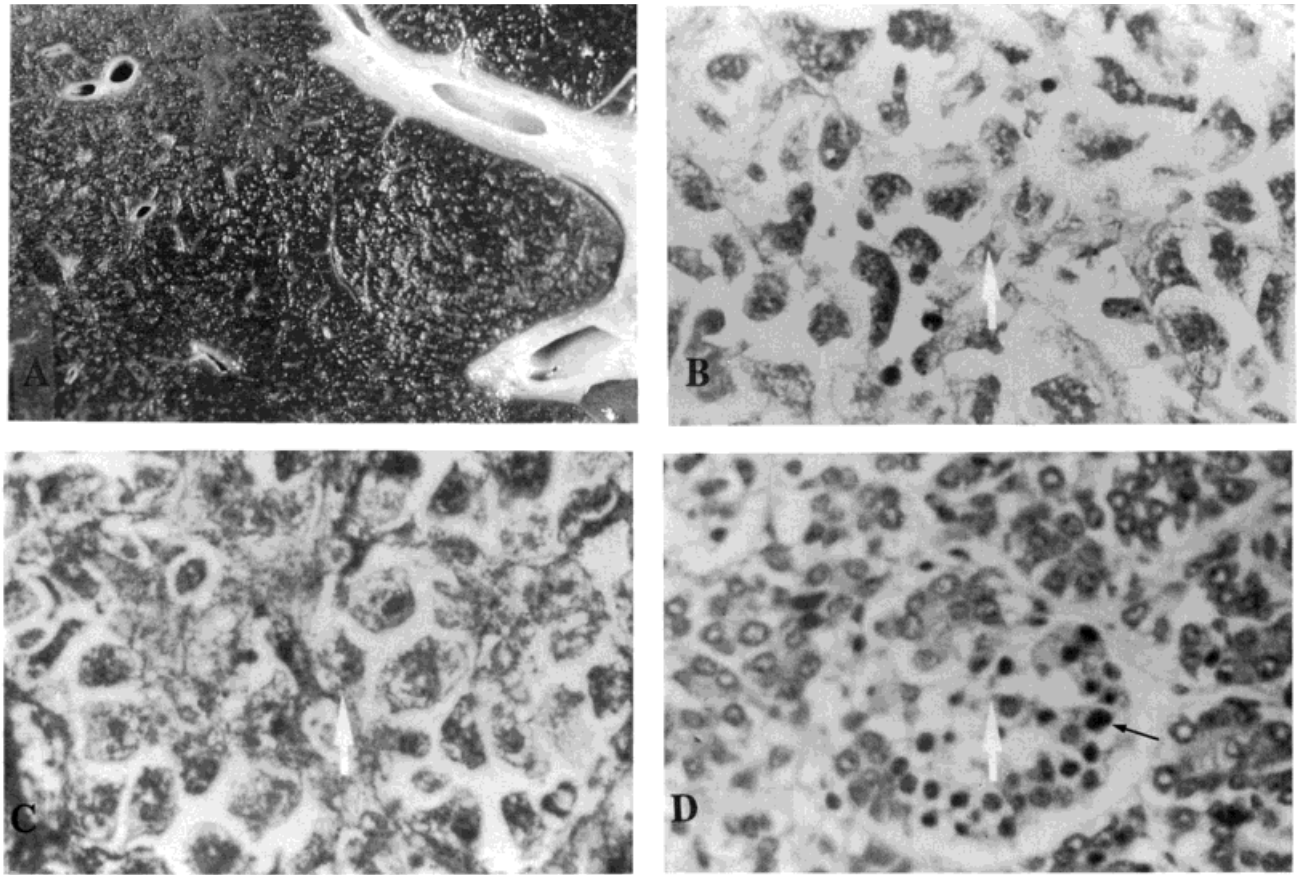

Fig. 2. Histological sections of liver and pancreas. **A**: Gross appearance of liver after formalin fixation showed dark green discoloration due to cholestasis. **B**: Distorted hepatic architecture with severe intrahepatic cholestasis and early septal fibrosis is seen (H&E, ×250). **C**: Enlarged hepatocytes filled with bile, (PAS, ×400). **D**: Islets of Langerhans, with cells containing enlarged hyperchromatic nuclei (H&E, ×250). The small black arrow indicates hyperchromatic nuclei.

Abnormalities were noted in several other tissues. Thymic sections showed marked depletion of thymocytes, consistent with a stressed thymus. Sections of the pancreas showed enlarged, hyperchromatic nuclei in the islet cells (Fig. 2D). Sections of the kidneys showed acute tubular necrosis. Hyperplastic adrenal glands with a prominent fetal cortex were evident. The left lung was unilobar and the right lung was bilobed. There was moderate bronchopneumonia.

BIOCHEMICAL STUDIES

In order to confirm the presumed diagnosis of SLO, analysis of tissue sterols was carried out to determine whether 7-dehydrocholesterol was present in elevated levels. A sample of frozen liver was saponified, extracted, and subjected to reverse-phase HPLC analysis. As shown in Table I, 7-dehydrocholesterol was present at a concentration of 2.31 mg $\times$ g^{-1}. Cholesterol was present at a concentration of 0.79 mg $\times$ g^{-1}. 8-dehydrocholesterol and 19-nor-5,7,9(10) cholestrien-3β-ol were also found. These were reported previously to be present in serum and tissues from SLO patients [Salen et al., 1995]. Sterol analysis was also carried out on tissue samples from several regions of the brain. In all cases, levels of 7-dehydrocholesterol were much higher than levels of cholesterol (Table I). A representative chromatogram is shown in Figure 4.

Since it is now established that SLOS patients have an impaired ability to synthesize cholesterol, it was of interest to determine whether their LDL receptor expression was increased in order to compensate. Sections of liver and brain from K.S. and an age- and sex-matched control were incubated with a 1:150 dilution of anti-LDL receptor sera [Ness and Zhao, 1994]. LDL receptor protein was detected by peroxidase staining. As shown in Figure 5, immunoreactive LDL receptor protein was readily detected in the liver and brain from the patient, and was essentially undetectable in control samples. Thus, the patient has increased expression of LDL receptors, likely in an effort to obtain cholesterol for tissue needs. However, very little cholesterol is normally present in the serum of SLOS patients.

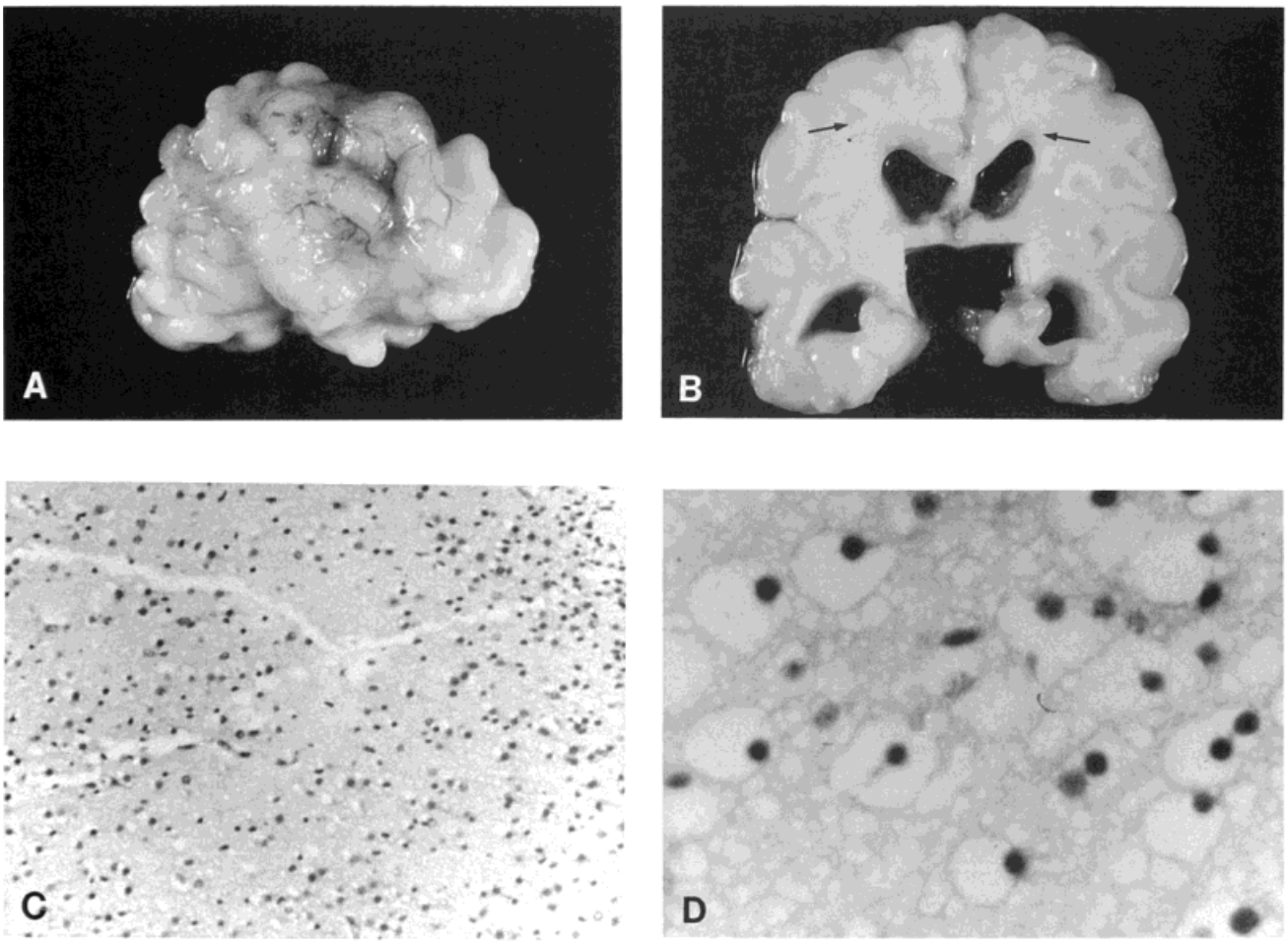

Fig. 3. Brain anomalies. **A**: Gross appearance of brain. The brain is small and has a severely abnormal gyral pattern. **B**: Coronal section of the brain, showing absence of corpus callosum, mild hydrocephalus, and small porencephalic cysts in the parietal cortex adjacent to the lateral ventricle. Kernicterus with billirubin staining of the basal ganglia was also noted. **C**: Abnormal neuronal migration with four instead of six cortical layers is evident in this microscopic section of the brain through the cortex (H&E, ×100). **D**: Severe lack of myelin is shown in this brain section (H&E, ×400).

DISCUSSION

The finding of profound hyperbilirubinemia in this SLO subject may be attributable to a lack of bile acids due to a severe deficiency in 7-dehydrocholesterol Δ^7 reductase activity. The high ratio of 7-dehydrocholesterol to cholesterol suggests that 7-dehydrocholesterol Δ^7 reductase may have been low in this subject. Attempts to measure enzyme activity were unsuccessful due to the poor quality of the tissue available for this determination. Since cholesterol serves as both a substrate and an inducer of cholesterol 7 α hydroxylase, the enzyme which catalyzes the rate-limiting step of bile acid biosynthesis [Ness et al., 1991], decreased hepatic cholesterol levels (Table I) would be expected to seriously impair bile acid synthesis. Insufficient production of bile acids would limit the infant's ability to eliminate bilirubin (indirect-acting). It would also explain the inability to feed that is seen frequently in SLOS patients. The severity of the clinical course in the present patient is consistent with this view. There has been a previous report of moderate conjugated hyperbilirubinemia (direct, 4.3–6.1 mg/dl; indirect, 2.2–2.7 mg/dl) in an infant with SLOS [Curry et al., 1987].

The current treatment for SLOS is to give 20–60 mg/kg per day of cholesterol either by mouth or via a gastrointestinal tube [Irons et al., 1995]. The goal of this treatment is to increase cholesterol levels in serum and other body fluids, and to decrease endogenous synthesis of 7-dehydrocholesterol. Since bile acids are needed for absorption of dietary lipids, these have been incorporated in some treatment protocols. They include ursodeoxycholic acid at 15 mg/kg per day and chenodeoxycholic acid at 7 mg/kg per day. Chenodeoxycholic acid also acts to suppress expression of the rate-limiting enzyme of cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme reductase. Thus, it would be expected to lower production of 7-dehydrocholesterol. This treatment protocol has produced modest results in most infants. In no case has the serum cholesterol even

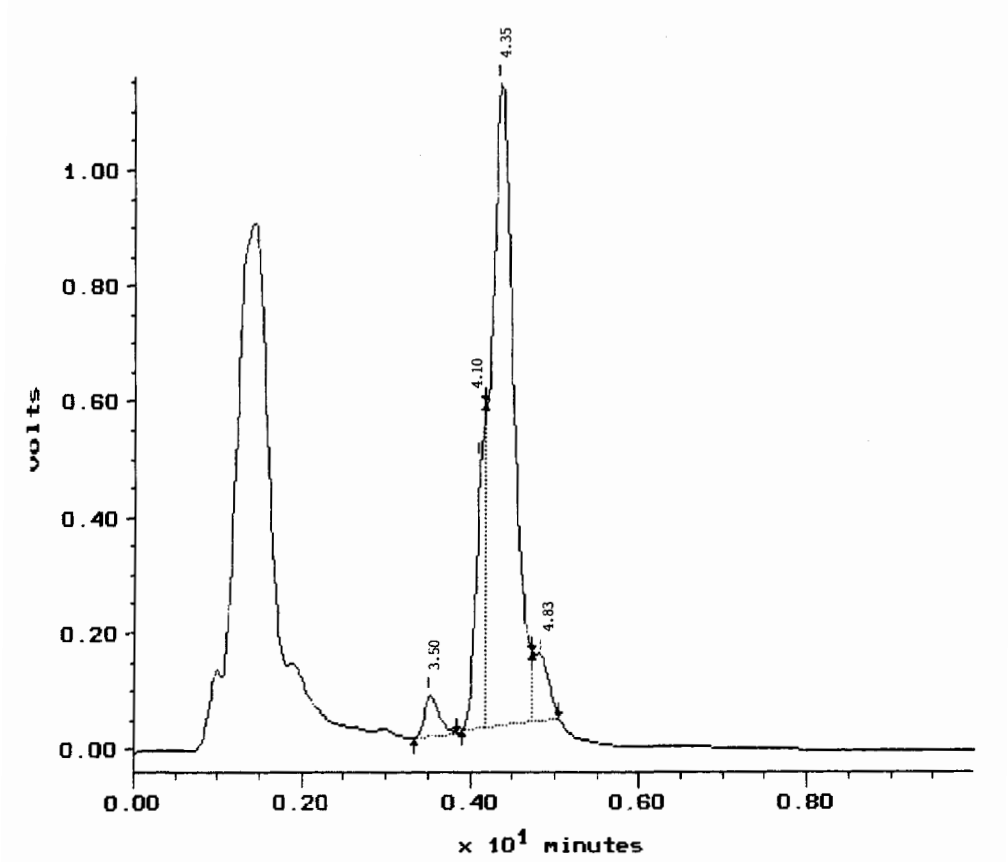

Fig. 4. Typical HPLC chromatogram of sterols. The peaks at 3.50, 4.10, 4.35, and 4.83 min represent 19-nor-5,7,9(10)cholestatrien-3β-ol, 8-dehyrocholesterol, 7-dehydrocholesterol, and cholesterol, respectively. Sterols were extracted from a sample of brain cortex from K.S.

been raised to near normal levels [Irons et al., 1995]. In the SLOS patient with the lowest initial serum cholesterol who was started the earliest on this treatment, a significant increase in serum cholesterol was observed. However, the level achieved was still <40 mg/dl.

The finding of increased LDL receptor expression in the present case suggests an alternative treatment approach. Perhaps serum lipoproteins could be infused. With the elevation in tissue LDL receptor levels, these lipoproteins could be readily taken up and used. This approach would obviate the inability of severely affected SLOS patients to absorb lipid-rich diets. Such treatment should raise hepatic cholesterol levels sufficiently to initiate bile-acid production, thereby enabling SLOS infants to begin to feed by mouth successfully. It has also been demonstrated that feeding cholesterol is the most effective way in which to lower 7-dehydrocholesterol levels in a rat model of SLOS [Xu et al., 1995]. Supplying all lipoproteins rather then just LDL may be desirable, since tissues such as heart and skeletal muscle express very low levels of LDL receptors but much higher levels of very low density lipoprotein (VLDL) receptors [Takahashi et al., 1992]. This concept of giving serum lipoproteins was recently tested in a severely affected SLO patient by infusing fresh-frozen plasma. Significant improvement was observed (Kelly, personal communication). In contrast with administration of serum lipoprotein, dietary cholesterol will be taken up predominately by the liver via the chylomicron remnant receptor pathway.

TABLE I. Tissue Sterol Levels*

	K.S.		Control	
Tissue	7DHC	Chol	7DHC	Chol
Midbrain	7.73	1.39		
Cerebellum	6.50	2.50		
Cortex	5.36	0.66		
Total brain	6.53	1.51	0.088	3.72
Total liver	2.31	0.79	0.016	2.55

*All values are presented as mg × g^{-1} of tissue. 7DHC, 7-dehydrocholesterol; Chol, cholesterol.

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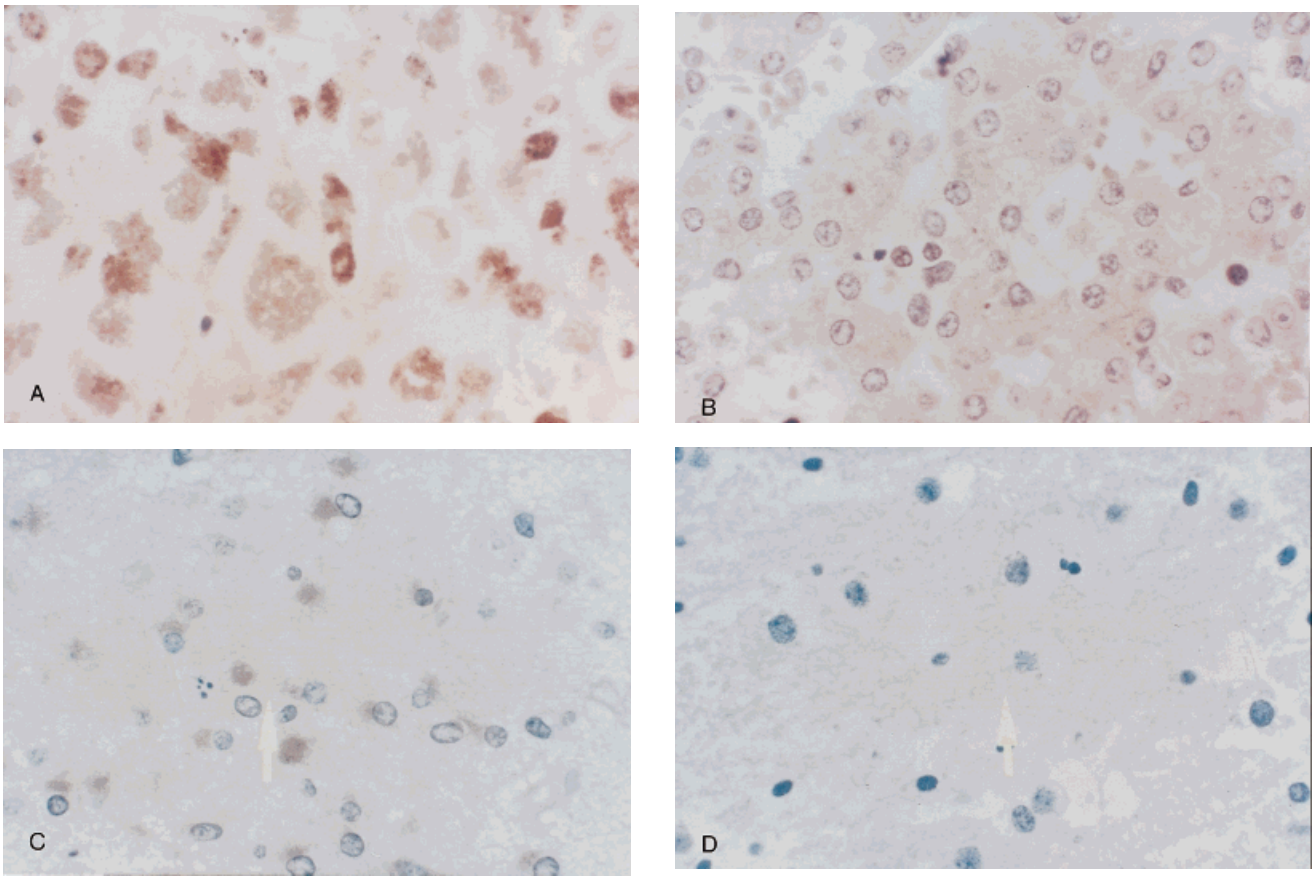

Fig. 5. Immunochemical staining of LDL receptor in liver and brain. **A**: Liver section from K.S. **B**: Liver section from an age- and sex-matched control. **C**: Brain section from K.S. **D**: Brain section from an age- and sex-matched control.

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